## REMARKS

### I. The Subject Matter of the Claims

In general, the subject matter of the claims relates to virulence genes of gram-negative bacteria, particularly of the family *Pasteurellaceae,* and immunogenic and vaccine compositions thereof.

### II. The Objections to the Specification

Paragraph five of the Office Action states that the amino acids disclosed in Table A at page 19, lines 25-32 and in Table B, lines 7-17 and 19 should be separated by commas to ensure they are not mistaken for peptide sequences.

The Applicants have amended the specification to include commas between the one letter amino acid abbreviations in order to ensure that the string of amino acids is not erroneously read as an amino acid sequence.

It is noted that the Applicants received notification in Paper 12, dated August 28, 2001, that the specification was not in sequence compliance. In preparing the correction, the above mentioned strings of amino acids disclosed in Tables A and B were inadvertently placed in the sequence listing as SEQ. ID NOs.: 166-169. The present amendment corrects this error. Applicants include herein a substitute page 262 in the sequence listing in which SEQ. ID NOs.: 166-169 are deleted, a computer readable format of the sequence listing reflecting this change, and statement under 37 CF.R. §1.821.

Paragraph five of the office action also points out that Table 1, Column LD50, pages 37-38, discloses numbers that are unclear when compared to the disclosure of the LD50 numbers in the text.

The numbers in Table 1, Column LD50 have been amended herein to clarify that they represent exponential numbers (i.e. 10E4 is $10^4$).

The objections to the specification may therefore be withdrawn.

## III. Amendments

Support for the amendment to claims 1 and 7 can be found at page 37, Table 1, which indicates that the attenuated bacterial strain comprises a mutation in the atpG protein coding region set out in SEQ. ID NO.: 3. Claim 1 is also amended to incorporate the limitation of decreased expression recited in dependent claim 2 as originally filed, and cancelled herein. Claim 31 is amended to appropriately reflect elected claims.

Pursuant to 37 C.F.R. §1.121, a marked-up version of the amendment to the specification and claims made herein is attached hereto as Appendix A. For the convenience of the Examiner, also attached, as Appendix B, is a copy of claims 1-24 and 31-33 as they read upon entry of the present amendment.

The amendment includes no new matter

## IV. Patentability Arguments

### A. The Enablement Rejection of Claims 1-24 and 31-33 under 35 U.S.C. § 112, First Paragraph, May Properly Be Withdrawn.

The Examiner rejected claims 1-24 and 31-33 for reciting subject matter assertedly not described in the specification in a manner sufficient to enable any person skilled in the art to make and use the invention commensurate in scope with the claims. The Examiner states: the specification does not reasonably disclose formulation of vaccines for "any" gram-negative bacteria that comprises "any" mutation in SEQ. ID NO.: 3 or a species homolog thereof for the induction of a protective response; the specification does not provide substantive evidence that the claimed vaccines are capable of inducing protective immunity utilizing bacteria that possess "any" level of decreased activity for the gene products of SEQ. ID NO.: 3; any mutation which does not attenuate the bacterium would result in induction of

an infection rather than protection; demonstration of an efficacious vaccine is required for the skilled artisan to be able to use the claimed inventions and reasonably predict the outcome of the administration of the claimed vaccine; and the specification fails to provide an adequate written description of all the species of SEQ. ID NO.: 3 that evidence attenuating mutations, and species homologs that would induce a protective immune response. The Applicants respectfully disagree.

The disclosure in the specification in combination with what was known in the art as of the priority date clearly provides more than sufficient guidance for the production of vaccine compositions derived from bacteria with mutations in the atpG gene that result in decreased expression of the gene atpG product, regardless of the strain of bacteria or the type of mutation that results in an attenuated bacteria with reduced expression of the atpG gene product. Moreover, in view of the detailed disclosure for making and using the exemplified embodiments, in combination with the knowledge in the art, the worker of ordinary skill can readily extrapolate the teachings in the specification to all aspects of the claimed subject matter with a high degree of predictability. Accordingly, every aspect of the claimed subject matter can be practiced without undue experimentation.

The specification expressly discloses two examples of bacterial atpG genes; the *P. multocida* gene in SEQ. ID NO.: 3 and the *A. pleuripneumonia* gene in SEQ. ID NO.: 132, which is a species homolog of SEQ. ID NO.: 3 as recited in the claims. Moreover, the art at the time the application was filed included the complete sequence for numerous other atpG genes from other gram negative bacteria, e.g. *E. coli* (Genbank Accession No. NP-290372, *Neisseria meningitidis* (Genbank Accession No. NP-283338), *H. influenzae* (Genbank Accession No. NP-438640), *Salmonella typhimurium* (Genbank Accession No. AF188265_7), *and Colwelia maris* (Genbank Accession No. BAB82483). The significance of these other "known" genes to bacterial virulence, however, was not known. Additionally,

techniques were known in the art by which any gram-negative species homologs of the atpG in SEQ ID NO: 3 could be identified, as described in the specification at page 11, lines 9 through 12. For example, the specification describes techniques such as Southern hybridization and PCR utilizing the atpG sequence of SEQ. ID NO.: 3 as a probe to screen chromosomal libraries of other gram-negative bacteria, also sets forth mutation complementation experiments as a means for identifying homologs of the atpG gene. The specification, in view of the art, therefore provides guidance for making and using "any" atpG gene to produce the invention.

The specification further teaches, for example, at page 9, lines 10 through 27, the method of signature tagged mutagenesis useful for induction of mutations in bacterial virulence genes, wherein mutations are produced by insertion of a tagged transposon element into the genome, and the mutated gene then recovered based on its unique signature tag. In addition to this method, at page 13, lines 9-19, the specification describes other methods for inducing mutations in any gene, wherein a desired sequence of DNA is replaced with a marker gene such as green fluorescent protein or luciferase. Moreover, techniques for inducing mutations were also well known in the art at the time of filing, such as chemical induction of frameshift mutations (Maenhaut-Michel G. et al, *Mol Gen Genet* 1992; 235:373-80), standard transposon mutagenesis (Grkovic S., *Mol Gen Genet* 1996. 250:323-8), and those described in *Current Protocols in Molecular Biology*, (Chapter 8, John Wiley and Sons, New York, 2000). Here again, the specification in view of the art fully enables making and using "any" mutation in an atpG coding region.

The specification further describes methods of determining levels of attenuation in mutated bacterial strains. Example 2, beginning on page 35, discloses methods for screening mutant gram-negative bacteria, exemplified by *P. multocida*, for attenuated virulence. At page 36, lines 23 through 27, the criteria for assessing an appropriate degree of

attenuation in bacterial strains is described, wherein a desirable mutant bacteria is one that shows significantly decreased viability in its host and as such is recoverable from its host at relatively low levels. Thus, any mutant demonstrated to be highly reduced in the recovery pool is a candidate vaccine. The worker of ordinary skill in the art would readily appreciate that the methods described can be used to assess the attenuation of any gram-negative bacteria, wherein attenuation results in a bacterial strain with decreased growth and infectivity rate, regardless of the site of mutation of the gene. Importantly, the specific activity of the mutated gene product does not need to be measured to actualize an attenuated strain of bacteria; it is only important that the mutation result in a strain that demonstrates decreased viability. Accordingly, the specification teaches all that is necessary to identify "any" bacteria as a potential vaccine, and these teachings clearly eliminate any bacterial strain that is not attenuated.

The specification further describes formulation of vaccine compositions in Example 3, page 37, lines 6 through 14, and discloses methods for challenge of animals previously vaccinated with the attenuated mutant bacteria produced and identified as described above. The specification demonstrates through challenge studies that these vaccine formulations give rise to protective immunity against wild type *P. Multocida* and *A. pleuripneumonia* infection in challenge animals. Example 3, Page 37, Table 1 indicates that SEQ. ID NO.: 3 of *P. multocida* induces protective immunity in ten out of ten mice challenged with wild type virus, while Example 11, beginning on page 52, and shown in Table 4, page 53, illustrates that vaccination with mutant A. *pleuripneumonia* atpG (SEQ. ID NO.: 132) resulted in survival of all animals (pigs) challenged with wild type bacteria. Thus, the specification discloses unequivocal evidence of effective protective immunity arising from claimed vaccine compositions, and this teaching can readily be applied to "any" vaccine composition to determine its effectiveness.

The combination of these teachings demonstrates the necessary techniques for making and using the invention as claimed and there is no reason to believe that the worker of ordinary skill in the art, with knowledge in the art available at the time the application was filed, could not apply these teachings to make and use every aspect of the invention as claimed without undue experimentation. The step-by step approach described in the specification reliably permits an assessment at every stage of producing the claimed bacteria and vaccine composition. As a result of this detailed description, failure at any step would indicate how and what type of changes should be made to arrive at a predictable result without any undue experimentation on the part of the worker of ordinary skill in the art.

In the Office Action, the Examiner relates several prior art instances of unpredictability in determining the effectiveness of a vaccine. The Applicants contend that the demonstration of an effective vaccination at page 37 of the specification using *P. multocida* which contain a mutation in SEQ. ID NO.: 3 renders the Examiner's citation of these references moot. While the Applicants note the Examiner's view that the art is replete with instances of ineffective vaccines, the specification clearly describes an effective method for inducing protective immunity against challenge with wild type bacteria by administration of the claimed mutant bacteria, thus avoiding the potential hazards of untested vaccines.

Because the specification explicitly describes attenuated strains of gram-negative bacteria, sets forth methods for isolating atpG encoding homologs and methods for introducing mutations in the gene product, discloses methods for measuring attenuation of bacterial strains, and demonstrates use of attenuated bacteria for the induction of protective immunity to subject animals upon vaccination, the Applicants submit that the specification is fully enabling for the entire scope of the subject matter of the claims and that the rejection under 35 U.S.C. §112, first paragraph, for asserted lack of enablement, should properly be withdrawn.

**B. The Written Description Rejection of Claims 1-33 under 35 U.S.C. §112, First Paragraph, May Properly Be Withdrawn.**

The Examiner rejects claims 1-33 under 35 U.S.C. §112, first paragraph, for assertedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor, at the time the application was filed, had possession of the invention. The Examiner states that while the specification discloses SEQ. ID NO.: 3 from *P. multocida* and SEQ. ID NO.: 132 from *A. pleuropneumoniae,* no other specific species homologs of the identified nucleic acid sequences are disclosed and that, with the exception of SEQ. ID NOs.: 3 and 132, the skilled artisan cannot envision the detailed structure of the polynucleotides encompassed by the claims. The Applicants respectfully disagree.

The Applicants contend that the Examiner has applied the written description analysis that is specifically applicable for nucleic acids and that this analysis is not appropriate for all "genus" subject matter. The claimed invention relates to attenuated gram-negative bacteria and vaccine compositions comprising the bacteria and not to polynucleotides *per se*.

For a genus that is not directed to, for example, polynucleotides, the written description guidelines indicate that where there is no substantial variation within a genus and where the Applicant conveys to one of skill in the art that he was in possession of the necessary common attributes and features of the members of the genus, a written description rejection is not proper. The Applicants submit that the specification meets this threshold.

The claims are directed to attenuated bacterial strains resulting from a mutation in the atpG gene. The specification clearly shows that mutations in atpG genes result in attenuation of the bacteria which is an effective vaccine. With this disclosure, the Applicants have fully described every common feature that every species in the claimed

genus must possess. There is no need to precisely define the chemical structure of every atpG gene in every bacteria or vaccine. Indeed, there is no need for such a description as evidenced by the fact that only attenuation of a strain (i.e. the end result) is important. The precise chemical change is irrelevant.

Thus, Applicants submit that the rejection should properly be withdrawn.

**C. The Rejection of Claims 1-24 and 31-33 under 35 U.S.C. §112, Second Paragraph, May Properly Be Withdrawn.**

The Examiner variously rejects claims 1-24 and 31-33 under 35 U.S.C. §112, second paragraph, as assertedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner also rejects these claims by stating that claims 1-24 and 31-33 recite non-elected inventions and therefore do not distinctly claim Applicants' invention. Each rejection will be addressed separately in a section below.

C.1. Objections to claims 1-24 and 31-33

The Examiner states that claims 1-24, 31-33 recite non-elected species and therefore do not distinctly claim Applicants' invention. The Examiner also states that claims 1-24 and 31-33 recite "a gene," and that the elected "gene" species ( SEQ. ID NO.: 3) contains two full open reading frames and a partial gene sequence, and not only one "gene" as stated in the claims.

The Applicants submit that the amendment to the claims to recite a mutation in the atpG protein coding region of SEQ. ID NO.: 3 obviates the Examiner's rejections.

C.2. Objections to claims 2, 8, 14, 20

The Examiner states that claims 2, 8, 14 and 20 recite the phrase "decreased expression of a gene product" and asks which of the three gene products does the claim refer. In a second rejection to these claims the Examiner contends that the claims depend from claims 1 and 7, requiring the bacterial mutation to result in decreased gene expression and decreased activity of a gene product. The Examiner asserts that the claims could require two mutations and that a mutation with decreased expression of a gene product does not necessarily constitute a mutation with decreased activity of a gene product.

The Applicants submit that the amendment to claims 1 and 7 to recite a mutation in the atpG protein coding region of SEQ. ID NO.: 3 obviates the Examiner's first rejection.

The Applicants respectfully disagree with the second rejection. While independent claim 7 does recite a mutation resulting in decreased activity of a gene product, the mutations described in the dependent claims are further limitations of the decrease in activity, not additional mutations. The Applicants contend that a decrease in expression of a gene product does reduce the overall activity of the gene product in some manner, be it inherent enzymatic activity or subsequent downstream activity. For example, a decrease in gene expression could result from a mutation which almost completely abolishes gene expression, thereby resulting in no gene product being produced and thus no activity. Alternatively, a decrease in gene expression could result from a reduced rate of transcription or translation of the gene, but also result in an essentially active gene product which retains the same specific activity as a wild type gene. However, by slowing the rate of transcription or translation of the gene product, the gene product will not function at the same capacity as its wild type counterpart. Thus, by altering the amount of gene product available as a result

of a decrease in gene expression, the gene product cannot retain the same observed, overall cellular activity levels that a wild type gene product would possess.

The Applicants submit that this rejection may properly be withdrawn.

C.3. Objections to claims 3, 9, 15, and 21

The Examiner states that claims 3, 9, 15 and 21 recite the phrase an "inactive gene product" and asks which gene product is inactive in light of the gene products encoded within SEQ. ID NO.: 3.

The Applicants submit that the amendment to claims 1 and 7 to recite a mutation in the atpG protein coding region of SEQ. ID NO.: 3 obviates the Examiner's rejection.

C.4. Objections to claims 4, 10, 16, 22

The Examiner states that claims 4, 10, 16, and 22 are dependent claims that recite the phrase "deletion of all or part of said gene," while the independent claims require the expression of the gene but with decreased activity. The Examiner asserts (1) that three gene products are encompassed by the claim, and (2) that with the complete deletion of the gene, the gene product would not be expressed as required by the independent claims.

The Applicants submit that the amendment to claims 1 and 7 to recite a mutation in the atpG protein coding region of SEQ. ID NO.: 3 obviates the Examiner's first rejection.

The Applicants respectfully disagree with the second rejection. Independent claim 7 recites decreased activity of a gene product encoded by the mutated gene and this decrease may arise from partial or complete deletion of a gene which results in either a decrease in or a complete absence of observed cellular activity of the encoded gene product.

The Applicants therefore contend that, a gene product does not necessarily need to be expressed to obtain decreased activity of the gene product, and submit that the rejection may properly be withdrawn.

C.5. Objections to claims 5, 11, 17, 23

The Examiner states that claims 5, 11, 17 and 23 are dependent claims that define the mutation as a deletion of a gene. The Examiner (1) asserts that the gene products encompassed by the claim are not clearly defined and (2) asks what activity the gene product would have if the gene deletion is a partial or complete deletion.

The Applicants submit that the amendment to claims 1 and 7 to recite a mutation in the atpG protein coding region of SEQ. ID NO.: 3 obviates the Examiner's first rejection.

The Applicants respectfully disagree with the second rejection. Partial (for example, 10%) or complete (99%) deletion of the gene can result in varying levels of gene product produced and the degree of alteration can have a dramatic effect on activity of the gene product. Regardless of the degree of change in activity, it is important to focus on the fact that the resulting bacteria must be attenuated. How the attenuation occurs is irrelevant (at least with respect to the degree of atpG mutation).

The Applicants submit that this rejection may properly be withdrawn.

C.6. Objections to claims 6, 12, 18, 24

The Examiner states that claims 6, 12, 18 and 24 are dependent claims and define the mutation to be an insertion in the gene, which may result in decreased expression of the gene product or the expression of an inactive gene product. The Examiner asks (1) which gene product is being inactivated, and (2) how, as in claim 2, decreased expression of a gene product leads to decreased activity of the same gene product.

The Applicants submit that the amendment to claims 1 and 7 to recite a mutation in the atpG protein coding region of SEQ. ID NO.: 3 obviates the Examiner's first rejection.

The Applicants respectfully disagree with the second rejection. An insertion which causes production of an inactive or practically inactive gene product effectively acts like any mutation, mimicking conditions as if less or no gene product were produced. The decrease (whole or partial) in active gene product results in a decrease or absence of overall activity within the cell.

The Applicants submit that this rejection may properly be withdrawn.

As set forth above, a mutation that results in decreased activity of a gene product can result from several different mechanisms, including decreased expression of a gene, expression of an inactive gene, deletion of all or part of a gene, or insertion in a gene. The decreased activity is manifest as a decreased overall observable cellular function of the gene product, not solely in the possible decreased specific activity of the gene product. Thus, the Applicant submits that, for the reasons stated above, the rejections of claims 2-6, 8-12, 14-18, and 20-24 under 35 U.S.C. §112, second paragraph, should properly be withdrawn.

C.7. Objections to claim 13

The Examiner states that claim 13 recites non-elected species as a result of election of SEQ. ID NO.: 3. The Applicants respectfully disagree. Claim 13 relates to gram-negative bacterial species that comprise species homologs of SEQ. ID NO.: 3 as claimed in claim 1. Evidence of genes homologous to SEQ. ID NO.: 3 species is outlined in the specification at page 41, lines 3 through 5, and as discussed herein others were known in the art. As such, the Applicants submit that the claim does not require amendment.

C.8. Objection to claims 31-33

The Examiner rejects claims 31-33 for depending from non-elected claims. The Applicants submit that the amendment to claim 31 to recite dependency to claims 1-24 obviates the Examiner's objection to the recitation of non-elected inventions in the claims.

**D. The Rejection of Claims 1-8, 12 and 31-32 under 35 U.S.C. §102(b), May Be Properly Withdrawn.**

The Examiner states that claims 1-5, 31-32 are rejected under 35 U.S.C. § 102(b), as assertedly being anticipated by the disclosure of Nakamoto and that claims 1-2, 6, 7-8, 12, 31-32 are rejected as assertedly being anticipated by the disclosure of Gwinn. The Examiner contends that Nakamoto discloses an ATP snythase gamma subunit deficient strain of *E. coli*, a gram negative bacteria, with mutations in the gamma subunit coding region which result in an ATPase with lower activity. The Examiner also contends that Gwinn discloses a mutant gram negative bacteria which is a species homolog of SEQ. ID NO.: 3 wherein the mutant evidences reduced activity of a gene product.

The Applicants submit that the amendments to the claims to recite a gram-negative bacteria possessing a mutation resulting in decreased expression of the encoded gene product obviates the Examiner's rejection based on Nakamoto. Nakamoto describes a mutated *E. coli* atpG gene which results in a decrease in ATPase activity, but does not address the protein expression levels resulting from the outlined mutation. As such, the subject matter of the rejected claim is not disclosed in Nakamoto, and the rejection under 35 U.S.C. §102(b), may properly be withdrawn.

Applicants asserts that Gwinn discloses a mutation in the atpA gene of gram-negative bacteria *H. influenzae* and that the subject matter of the amended claims relates to the atpG protein coding region of SEQ. ID NO.: 3. Because Gwinn specifically recites mutations only in the atpA gene, the mutated gene is not a species homolog of the atpG gene

(SEQ. ID NO.: 3) recited in the claims and thus, this rejection under 35 U.S.C. §102(b), may properly be withdrawn.

**V. CONCLUSION**

In view of the amendments and remarks made herein, Applicants respectfully submit that claims 1-24 and 31-33 are in condition for allowance and respectfully request expedited notification of same.

Respectfully submitted,

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APPENDIX A

Version marked to show changes

In the specification

At page 19, lines 22 through 32:

**Table A**

**Conservative Substitutions I**

| SIDE CHAIN CHARACTERISTIC | | AMINO ACID |
|---|---|---|
| Aliphatic | Non-polar | G, A, P<br>I, L, V |
| | Polar - uncharged | C, S, T, M<br>N, Q |
| | Polar - charged | D, E<br>K, R |
| Aromatic | | H, F, W, Y |
| Other | | N, Q, D, E |

At page 21, lines 1 through 19:

**Table B**
**Conservative Substitutions II**

| SIDE CHAIN CHARACTERISTIC | AMINO ACID |
|---|---|
| Non-polar (hydrophobic) | |
| A. Aliphatic: | A, L, I, V, P |
| B. Aromatic: | F, W |
| C. Sulfur-containing: | M |
| D. Borderline: | G |
| Uncharged-polar | |
| A. Hydroxyl: | S, T, Y |
| B. Amides: | N, Q |
| C. Sulfhydryl: | C |
| D. Borderline: | G |
| Positively Charged (Basic): | K, R, H |
| Negatively Charged (Acidic): | D, E |

At page 37, line 25 through page 38, line 35:

Table 1
*P. multocida* Virulence Genes

| Nucleotide SEQ ID NO: | Representative Isolate | PossibleGene Function | Vaccination # survivors/total | Challenge # survivors/total | $LD_{50}$ |
|---|---|---|---|---|---|
| – | wild type | – | 0/10 | – | <10 |
| 23 | PM1B1 | guaB | 10/10, 10/10, 10/10 | 9/10, 9/10 | 4.3 x 10E6 |
| 11 | PM1D1 | dsbB | 10/10, 5/10 | 10/10, 5/5 | 8.4 x 10E4 |
| 3 | PM1BD7 | atpG | 5/5, 10/10 | 10/10 | >3 x 10E5 |
| 74 | PM1BE11 | yhcJ (HI0145) | 10/10 | 5/10 | >2 x 10E5 |
| 70 | PM1BF6 | yabK (HI1020) | 3/5, 8/10 | 9/9 | >2 x 10E5 |
| 19 | PM2G8 | fhaC | 4/5, 9/10 | 9/9 | >4 x 10E5 |
| 76 | PM3C9 | yiaO (HI0146) | 3/5 | | >6 x 10E5 |
| 118 | PM3G11 | UnkO | 4/5, 10/10 | 10/10 | >3 x 10E5 |
| 31 | PM7B4 | iroA (UnkB) | 0/5 | | |
| 17 | PM4C6 | fhaB (fhaB2) | 2/5, 10/10, 9/10 | 10/10, 9/9 | >3 x 10E6 |
| 89 | PM4G10-T9 | dnaA | 4/5 | | >5 x 10E5 |
| 1 | PM4D5-T5 | atpB | 5/5 | | >4 x 10E5 |
| 53 | PM4D5-T1 | UnkC2 | 5/5 | | >4 x 10E5 |
| 15 | PM4F2 | fhaB (fhaB1) | 3/5, 6/10, 10/10 | 6/6, 10/10 | >3 x 10E5 |
| 41 | PM5F7 | mreB | 4/5 | | 1 x 10E3 |
| 7 | PM5E2 | devB | 0/5, 3/10 | 2/3 | ? |
| 68 | PM6H5-T1 | xylA | 5/5 | | >3 x 10E5 |
| 78 | PM6H8 | yigF (HI0719) | 5/5, 9/10 | 9/9 | >3 x 10E5 |
| 108 | PM7D12 | pnp | 5/5, 9/10 | 9/9 | |
| 51 | PM8C1R1-T2 | UnkC1 | 5/5 | | ~6 x 10E5 |
| 37 | PM8C1-T3 | mglB | 5/5 | | ~6 x 10E5 |
| 58 | PM8C1R1-T6 | UnkD1 | 5/5 | | ~6 x 10E5 |
| 45 | PM10H7 | purF (HI1207) | 3/5, 8/10, 8/10 | 8/8, 8/8 | >3 x 10E5 |
| 25 | PM10H10-T2 | HI1501 | 5/5 | | >1 x 10E4 |
| 72 | PM11G8-T2 | ygiK | 5/5 | | >2.4 x 10E3 |
| 21 | PM11G8-T4 | greA | 5/5 | | >2.4 x 10E3 |
| 84 | PM12H6 | yyam (HI0687) | 3/5, 0/10 | | ~2.2 x 10E3 |
| 33 | PM15G8-T2 | kdtB | 5/5 | | >1.2 x 10E5 |
| 116 | PM15G8-T1 | UnkK | 5/5 | | >1.2 x 10E5 |
| 104 | PM16G11-T1 | hmbR | 3/5 | | >1.9 x 10E5 |
| 29 | PM16G11-T2 | hxuC | 3/5 | | >1.9 x 10E5 |
| 35 | PM16H8 | lgtC | 5/5, 10/10 | 10/10 | >2.4 x 10E5 |
| 80 | PM16H3 | yleA (HI0019) | 5/5, 10/10 | | > 2.0 x 10E5 |
| 49 | PM17H6-T1 | sopE | 4/5 | | ~6 x 10E5 |
| 120 | PM17H6 | UnkP | 4/5 | | ~6 x 10E5 |
| 5 | PM18F5-T8 | cap5E | 5/5 | | >2.4 x 10E5 |
| 82 | PM18F5-T10 | yojB (HI0345) | 5/5 | | >2.4 x 10E5 |
| 13 | PM19A1 | exbB | 5/5, 10/10 | 10/10 | >1.2 x 10E5 |
| 112 | PM19D4 | rci | 5/5, 8/10 | 8/8 | ~1.6 x 10E5 |
| 39 | PM20A12 | mioC (HI0669) | 3/5, 8/10 | 8/8 | ~2 x 10E4 |
| 60 | PM20C2 | UnkD2 | 5/5, 10/10 | 10/10 | >8.2 x 10E6 |

In the Claims

1. ~~A~~ An attenuated gram-negative bacteria comprising a mutation in the atpG protein coding region ~~a gene represented by a nucleotide sequence~~ set forth in ~~any one of~~ SEQ ID NOs: ~~1,~~ 3, ~~7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 163, and 164,~~ or a species homolog thereof, said mutation resulting in decreased ~~activity~~ expression of a gene product encoded by the mutated gene.

7. An attenuated *Pasteurellaceae* bacteria comprising a mutation in the atpG protein coding region ~~a gene represented by a nucleotide sequence~~ set forth in ~~any one of~~ SEQ ID NOs: ~~1,~~ 3, ~~7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 29, 31, 33, 37, 39, 41, 51, 53, 55, 57, 58, 60, 68, 70, 72, 74, 76, 78, 80, 82, 84, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 135, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156,~~

~~158, 160, 162, 163, and 164,~~ or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

31. An immunogenic composition comprising the bacteria according to any one of claims 1 through ~~30~~24.

APPENDIX B

1. (Amended) An attenuated gram-negative bacteria comprising a mutation in the atpG protein coding region set forth in SEQ ID NO: 3 or species homologs thereof, said mutation resulting in decreased expression of a gene product encoded by the mutated gene.

3. The gram-negative bacteria of claim 1 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

4. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of all or part of said gene.

5. The gram-negative bacteria of claim 1 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

6. The gram-negative bacteria of claim 1 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

7. (Amended) An attenuated *Pasteurellaceae* bacteria comprising a mutation in the atpG protein coding region set forth in SEQ ID NO.: 3 or a species homolog thereof, said mutation resulting in decreased activity of a gene product encoded by the mutated gene.

8. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

9. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

10. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in deletion of all or part of said gene.

11. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

12. The *Pasteurellaceae* bacteria of claim 7 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

13. The *Pasteurellaceae* bacteria of claim 7 selected from the group consisting of *Pasteurella haemolytica*, *Pasteurella multocida, Actinobacillus pleuropneumoniae* and *Haemophilus somnus*.

14. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

15. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

16. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of all or part of said gene.

17. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

18. The *Pasteurellaceae* bacteria of claim 13 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

19. The attenuated *Pasteurellaceae* bacteria of claim 13 that is a *P. multocida* bacteria.

20. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in decreased expression of a gene product encoded by the mutated gene.

21. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in expression of an inactive gene product encoded by the mutated gene.

22. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in deletion of all or part of said gene.

23. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in deletion of at least about 10%, at least about 20%, at least about 30%, at least about 40% at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% of said gene.

24. The *Pasteurellaceae* bacteria of claim 19 wherein said mutation results in an insertion in the gene, said insertion causing decreased expression of a gene product encoded by the mutated gene and/or expression of an inactive gene product encoded by the mutated gene.

31. (Amended) An immunogenic composition comprising the bacteria according to any one of claims 1 through 24.

32. A vaccine composition comprising the immunogenic composition according to claim 31 and a pharmaceutically acceptable carrier.

33. The vaccine composition according to claim 32 further comprising an adjuvant.

<210> 165
<211> 207
<212> PRT
<213> Actinobacillus pleuropneumoniae

<400> 165

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Met | Gln | Glu | Leu | Thr | Pro | Gln | Met | Trp | Gly | Leu | Val | Gly | Thr | Ser | Thr |
| 1 | | | | 5 | | | | 10 | | | | | 15 | | |
| Leu | Glu | Thr | Leu | Tyr | Met | Gly | Phe | Ala | Ala | Thr | Leu | Leu | Ala | Val | Val |
| | | 20 | | | | | 25 | | | | | 30 | | | |
| Val | Gly | Leu | Pro | Ile | Gly | Phe | Leu | Ala | Phe | Leu | Thr | Gly | Lys | Gly | Glu |
| | 35 | | | | | 40 | | | | | 45 | | | | |
| Ile | Leu | Glu | Asn | Pro | Arg | Leu | His | Gln | Val | Leu | Asp | Val | Ile | Ile | Asn |
| 50 | | | | | 55 | | | | | 60 | | | | | |
| Ile | Gly | Arg | Ser | Val | Pro | Phe | Ile | Ile | Leu | Leu | Val | Val | Leu | Leu | Pro |
| 65 | | | | 70 | | | | | 75 | | | | | 80 | |
| Phe | Thr | Arg | Leu | Leu | Val | Gly | Thr | Thr | Leu | Gly | Thr | Thr | Ala | Ala | Ile |
| | | | 85 | | | | | 90 | | | | | 95 | | |
| Val | Pro | Leu | Ser | Val | Ser | Ala | Ile | Pro | Phe | Phe | Ala | Arg | Leu | Thr | Ser |
| | | 100 | | | | | 105 | | | | | 110 | | | |
| Asn | Ala | Leu | Leu | Glu | Ile | Pro | Ala | Gly | Leu | Thr | Glu | Ala | Ala | Lys | Ser |
| | 115 | | | | | 120 | | | | | 125 | | | | |
| Met | Gly | Ala | Thr | Asn | Trp | Gln | Val | Val | Ser | Lys | Phe | Tyr | Leu | Pro | Glu |
| 130 | | | | | 135 | | | | | 140 | | | | | |
| Ser | Leu | Pro | Ile | Leu | Ile | Asn | Gly | Ile | Thr | Leu | Thr | Leu | Val | Ala | Leu |
| 145 | | | | 150 | | | | | 155 | | | | | 160 | |
| Ile | Gly | Tyr | Ser | Ala | Met | Ala | Gly | Ala | Val | Gly | Gly | Gly | Gly | Leu | Gly |
| | | | 165 | | | | | 170 | | | | | 175 | | |
| Asn | Leu | Ala | Ile | Ser | Tyr | Gly | Glu | His | Arg | Asn | Met | Val | Tyr | Val | Lys |
| | | 180 | | | | | 185 | | | | | 190 | | | |
| Trp | Ile | Ser | Thr | Ile | Ile | Ile | Val | Ala | Ile | Val | Met | Ile | Ser | Gln | |
| | 195 | | | | | 200 | | | | | 205 | | | | |